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Determination of Bioactive Components of *Annona squamosa* L Leaf by GC- MS Analysis

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ABSTRACT
Annona squamosa Linn, commonly known as Sugar apple, belonging to the family Annonaceae, is said to show varied medicinal effects, including insecticide, antiovulatory and abortifacient. Hence the present investigation was carried out to determine the chemical composition of *Annona squamosa* leaf extract using Gas Chromatography–Mass Spectrometry technique, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC-MS analysis of *Annona squamosa* leaf extract revealed the existence of Sodium benzoate (27.50%), 4, 4-Tert- Butylcalix(4)arene (12.34 %), 4, 4- Dimethylcholesterol (10.30%), Butyloctylpthalate (9.67%), stigmasterol acetate (2.92%), isoamylacetyate (2.29%) justifying the use of this plant to treat many ailments in folk and herbal medicine.

Keywords: *Annona squamosa,* GC-MS technique, Herbal medicine, Sodium benzoate.

INTRODUCTION

Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties. [1] Distinguished examples of these compounds include flavonoids, phenols, phenolic glycosides, saponins and cyanogenic glycosides. [2-3] Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as sources of antibiotic prototypes. [4-5] It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. [6]

Annona squamosa Linn. (Family; Annonaceae) commonly known as custard apple, is a woody, semi deciduos tree grown throughout India in rocky terrain with shallow and well drained soils. A bark decoction of this plant is used to prevent diarrhoea, while the root is used in the treatment of dysentery. A decoction of the leaves is used for cold and to clarify urine. Leaves are used to treat hysteria and fainting spells. The fruits of *Annona* are haematinic, cooling, sedative, stimulant, expectorant and maturant tonic. They are useful in treating anemia and burning sensation. The seeds are abortifacient and insecticidal and are useful in destroying lice in the hair. Fruit is used in making of ice creams and milk beverages. The bark and leaves contain annonaine, an alkaloid which is found to posses many of these properties. [7] Hypoglycemic and antidiabetic effect of *Annona squamosa* was reported in the leaf extract. [8-9] From the bark of *Annona squamosa*, a bioactive acetogenin with anticancer activity have been isolated. [10-11] Flavonoids from leaves [12] Aporphine alkaloids [13], glycoside [14] and squamoline were isolated from this plant. In the ayurvedic system of medicine, herbal extracts but not purified compounds have been used from centuries, because many constituents with more than one mechanism of action are considered to be beneficial. Since there is no relevant report on the phytoconstituents of *Annona squamosa* leaf it was chosen for the study.

This study was initiated to determine the compounds present in the leaves of *Annona squamosa* with the help of GC-MS technique, which may reveal an insight in its use in folk medicine.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Annona squamosa* plant was collected locally during the month of November to January. The taxonomic

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identification of these plant materials were authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai. (PARC/2009/456).

Preparation of leaf extract

Air dried powder was macerated with 100 mL ethanol and stored for 72 hrs in ice cold condition. After 72 hrs the extracts were filtered through a Whatmann filter paper No. 42 (125 mm) and the organic layer was allowed to evaporate The resulted dark green extracts were concentrated using a rotary evaporator with a water bath set at 40°C. The concentrated crude extracts were lyophilized into paste (5 and 15 g respectively) and used for further analysis.

Column Chromatography

10 g of the crude extract was subjected to column chromatography over silica gel (100-200 mesh) and eluted with 100% chloroform. The polarity of mobile phase was gradually increased from chloroform, ethyl acetate and methanol respectively. The ethyl acetate fraction of the *Annona squamosa* leaf extract was taken for GC-MS analysis.

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

GC-MS technique was used in this study to identify the phytocomponents present in the *Annona Squamosa* leaf extract. GC-MS technique was carried out at Sargam laboratory, Chennai, Tamil Nadu. GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0m, Diameter : 0.25 mm, Film thickness : 0.25 µm Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 2µl was employed. Injector temperature was 200°C and Ion-source temperature was 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver. 2.53.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The Name, Molecular weight, Molecular formula and Structure of the component of the test material was ascertained.

Table 1: Percentage yield of different fractions.

Fractions	Solvent	Obtained weight of fractions	Percentage of yield
Fr-1	Chloroform 100%	300mg	5.0
Fr-2	Chloroform:ethyl acetate 75:25	320mg	5.12
Fr-3	Chloroform:ethyl acetate 50:50	550mg	8.8
Fr-4	Ethyl acetate100%	900mg	14.4
Fr-5	Ethylacetate: Methanol 75:25	950mg	15.2
Fr-6	Ethylacetate:Methanol 50:50	1000mg	16
Fr-7	Methanol 100%	1400 mg	22.4

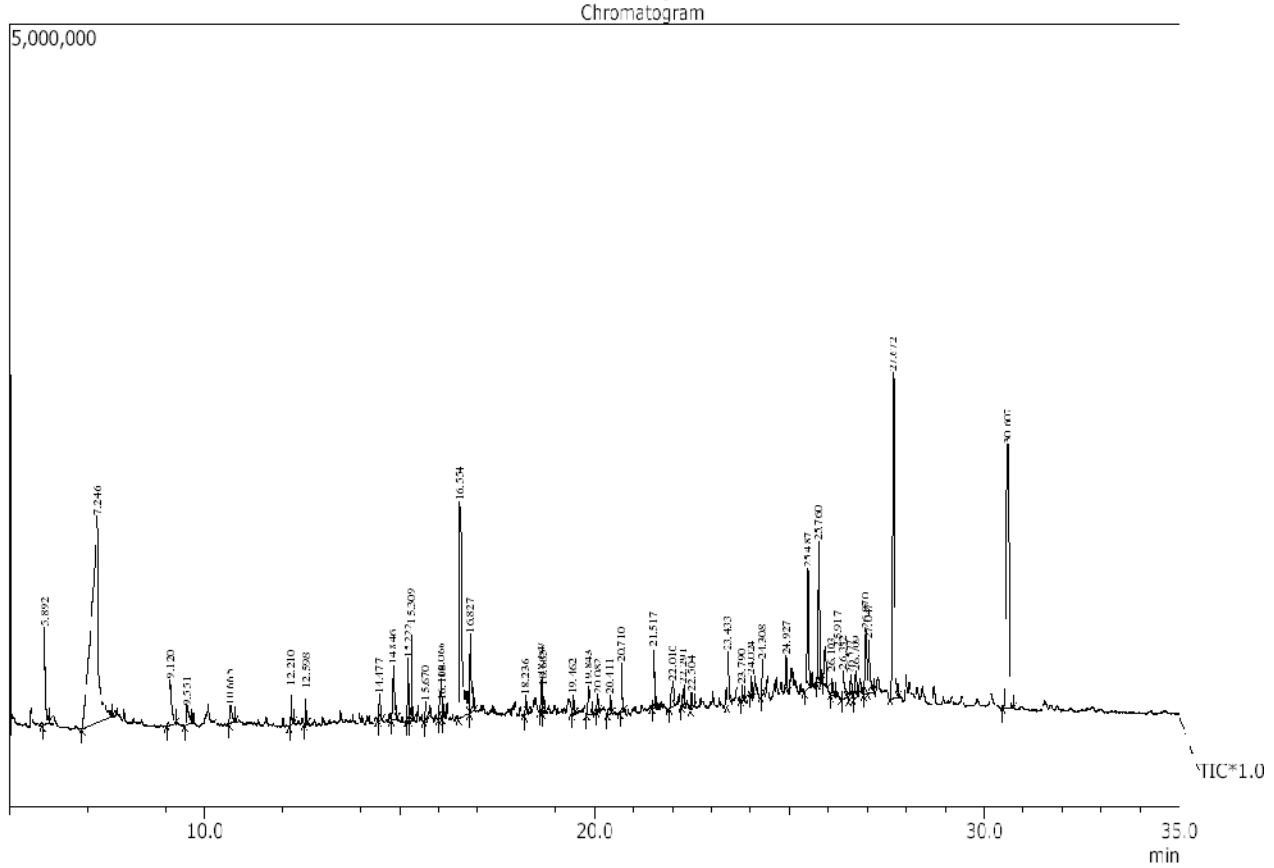

Fig. 1: Chromatogram obtained for ethyl acetate fraction of *Annona squamosa* leaf extract

Table 2: Total ionic chromatogram showing the compounds of ethyl acetate fraction of *Annona squamosa* leaf extract, retention time concentration and name of the compound.

PEAK	R.TIME	AREA	AREA%	NAME OF THE COMPOUND
1	5.892	1577943	2.29	Isoamyl acetate
2	7.246	18979384	27.50	Sodiumbenzoate
3	9.120	1342082	1.94	3-Ethoxypropanol
4	9.551	511871	0.74	Ethyl 2,3-epoxybutyrate
5	10.665	438885	0.64	Cinnamic acid
6	12.210	579085	0.84	n-Dodecanoic acid
7	12.598	403683	0.58	Diethyl Phthalate
8	14.477	373515	0.54	Myristic acid
9	14.846	986962	1.43	4,4,8-Trimethyl-non-7-en-2-one
10	15.222	689282	1.00	Neophytadiene
11	15.309	1174489	1.70	6,10,14Trimethylpentadecan-2-one
12	15.670	265114	0.38	(2E)-3,7,11,15-Tetramethyl-2-Hexadecene-1-ol
13	16.066	768727	1.11	7,9-Di-tert-butyl-1oxaspiro[4.5]deca-6,9-diene-2,8-dione
14	16.108	174913	0.25	1-Docosanol
15	16.554	6671185	9.67	Butyl octyl phthalate
16	16.827	1354234	1.96	Ethyl Hexadecanoate
17	18.236	221879	0.32	Hexadecanal Dsiallyl Acetal
18	18.647	517764	0.75	5,9-Dimethyl-2-(1-methylethylidene)cyclodecanol
19	18.683	414460	0.60	Ethyl stearate
20	19.462	262966	0.38	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3ol
21	19.843	734731	1.06	Cis-9-octadecenal
22	20.082	205574	0.30	5-Methyl-5-(4,8,12-trimethyltridecyl)dihydro-2(3H)- furanone
23	20.411	336459	0.49	n-Tetratriacontane
24	20.710	527831	0.76	Stearaldehyde
25	21.517	1071061	1.55	Palmitaldehyde
26	22.010	718168	1.04	Longifolenaldehyde
27	22.291	392798	0.57	Pentadecanal-
28	22.504	216190	0.31	2,3-Bis(Acetyloxy)Propyl Palmitate
29	23.433	980845	1.42	Oxalic acid, butyl 2-isopropylphenyl ester
30	23.790	235393	0.34	3-Isopropyl-1,2,3,3A,4,5,7,12Octahydrocyclopenta[D]anthracene-6,8,11(6AH)-trione
31	24.024	195094	0.28	delta.5-Ergostenol
32	24.308	441889	0.64	18-Oxokauran-17-ylacetate
33	24.927	375342	0.54	Dicyclooctanopyridazin
34	25.487	2018056	2.92	Stigmasterol acetate
35	25.760	1957977	2.84	Butyramide, 3-(3,3-dimethyl-3,4-dihydro-2H-isoquinolin-1-ylidene)-N-ethyl-2,4-dioxo-4-(piperidin-1-yl)
36	25.917	642436	0.93	11.Beta.,17.alpha.,21-trihydroxypregn-4-ene-3,20-dione N-Butyl Boronate
37	26.103	407110	0.59	Cyanazine
38	26.382	760232	1.10	Oridonin Oxide
39	26.577	445512	0.65	2-Chloro-4-Etoxy-6-Piperidine1-yl-[1,3,5]Triazine
40	26.709	534632	0.77	Octahydro-1H-Indene
41	26.970	1246672	1.81	Propionic acid, 3-benzoylamino-3-(4-propoxyphenyl)-
42	27.047	1229781	1.78	3-O-Acetyl-delta24-cycloartenol
43	27.672	7108827	10.30	4, 4-Dimethylcholesterol
44	30.607	8515796	12.34	4-tert-Butylcalix[4]arene

RESULTS AND DISCUSSION

Column Chromatography

Total of 51 fractions were collected from column and they were pooled to seven fractions according to their polarity. The details of collected fractions were given in table 1. 86.52 % of total extract was recovered and 13.48% of extract was lost. Chloroform and methanol did not elute much of the compounds. The ethyl acetate fraction of the *Annona squamosa* leaf was taken for GC-MS analysis.

GC-MS analysis

Forty-four compounds were identified in the ethyl acetate fraction of *Annona squamosa* leaf extract by GC-MS analysis. The chromatogram obtained by ethyl acetate fraction of *Annona squamosa* leaf extract was shown in Fig. 1.The active principle, area of the peak, Concentration (%) and Retention Time (RT) are presented in Table 2. The prevailing compounds were sodium benzoate (27.50%), 4, 4-Tert- Butylcalix(4)arene (12.34 %),4, 4- Dimethylcholesterol (10.30%), Butyloctylpthalate (9.67%), stigmasterol acetate (2.92%), isoamylacetyate (2.29%). The present investigation clearly indicates the highest percentage of sodium benzoate in ethylacetate fraction of *Annona squamosa* leaf extract. Sodium benzoate was used as an antifungal agent, antibacterial agent, as CNS stimulant and as a diagnostic aid in liver function tests (Dr. Duke's Phytochemical and Ethnobotanical Database).

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